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(54) Title: NOVEL HUMAN PROTEINS AND POLYNUCLEOTIDES ENCODING THEM

(57) Abstract

The present invention provides novel isolated SECX polynucleotides and the membrane-associated or secreted polypeptides encoded by the SECX polynucleotides. Also provided are the antibodies that immunospecifically bind to a SECX polypeptide or any derivative, variant, mutant or fragment of the SECX polypeptide, polynucleotide or antibody. The invention additionally provides methods in which the SECX polypeptide, polynucleotide and antibody are utilized in the detection and treatment of a broad range of pathological states, as well as to other uses.

ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify SECX variants (Arkin and Yourvan (1992) *PNAS* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6:327-331).

In one embodiment, cell based assays can be exploited to analyze a variegated SECX library, *e.g.*, a library of mutant SECX polypeptides. For example, a library of expression vectors can be transfected into a cell line that ordinarily responds to a particular ligand or receptor in a SECX-dependent manner, e.g., through a signaling complex. The transfected cells are then contacted with the putative SECX interactant and the effect of expression of the mutant SECX on signaling by the signaling complex can be detected, *e.g.* by measuring a cellular activity or cell survival. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of, *e.g.*, cytokine induction, and the individual clones further characterized.

Anti-SECX antibodies

The invention encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to any of the polypeptides of the invention.

An isolated SECX protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind SECX using standard techniques for polyclonal and monoclonal antibody preparation. The full-length SECX protein can be used or, alternatively, the invention provides antigenic peptide fragments of SECX for use as immunogens. The antigenic peptide of SECX comprises at least 4 amino acid residues of the amino acid sequence shown in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30 and encompasses an epitope of SECX such that an antibody raised against the peptide forms a specific immune complex with SECX. Preferably, the antigenic peptide comprises at least 6, 8, 10, 15, 20, or 30 amino acid residues. Longer antigenic peptides are sometimes preferable over shorter antigenic peptides, depending on use and according to methods well known to someone skilled in the art.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of SECX that is located on the surface of the protein, *e.g.*, a hydrophilic region. A hydrophobicity analysis of the human SECX protein sequence will indicate which regions of a SECX polypeptide are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, *e.g.*, Hopp and Woods, 1981, *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle 1982, *J. Mol. Biol.* 157: 105-142, each incorporated herein by reference in their entirety.

As disclosed herein, SECX protein sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active

portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen, such as SECX. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} and $F_{(ab')2}$ fragments, and an F_{ab} expression library. In a specific embodiment, antibodies to human SECX proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to a SECX protein sequence, or derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, or a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, recombinantly expressed SECX protein or a chemically synthesized SECX polypeptide. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*, or similar immunostimulatory agents. If desired, the antibody molecules directed against SECX can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of SECX. A monoclonal antibody composition thus typically displays a single binding affinity for a particular SECX protein with which it immunoreacts. For preparation of monoclonal antibodies directed towards a particular SECX protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (see Kohler & Milstein, 1975 *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (see Kozbor, *et al.*, 1983 *Immunol Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, *et al.*, 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (see Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Each of the above citations are incorporated herein by reference in their entirety.

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to a SECX protein (see *e.g.*, U.S. Patent No. 4,946,778). In addition, methodologies can be adapted for the construction of F_{ab} expression libraries (see *e.g.*, Huse, *et al.*, 1989 *Science* 246:

1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for a SECX protein or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. See *e.g.*, U.S. Patent No. 5,225,539. Antibody fragments that contain the idiotypes to a SECX protein may be produced by techniques known in the art including, but not limited to: (*i*) an $F_{(ab')2}$ fragment produced by pepsin digestion of an antibody molecule; (*ii*) an F_{ab} fragment generated by reducing the disulfide bridges of an $F_{(ab')2}$ fragment; (*iii*) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (*iv*) F_v fragments.

Additionally, recombinant anti-SECX antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent Application No. 171,496; European Patent Application No. 173,494; PCT International Publication No. WO 86/01533; U.S. Pat. No. 4,816,567; U.S. Pat. No. 5,225,539; European Patent Application No. 125,023; Better *et al.*(1988) *Science* 240:1041-1043; Liu *et al.* (1987) *PNAS* 84:3439-3443; Liu *et al.* (1987) *J Immunol.* 139:3521-3526; Sun *et al.* (1987) *PNAS* 84:214-218; Nishimura *et al.* (1987) *Cancer Res* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988) *J Natl Cancer Inst* 80:1553-1559); Morrison(1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J Immunol* 141:4053-4060. Each of the above citations are incorporated herein by reference in their entirety.

In one embodiment, methodologies for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of a SECX protein is facilitated by generation of hybridomas that bind to the fragment of a SECX protein possessing such a domain. Antibodies that are specific for an above-described domain within a SECX protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

Anti-SECX antibodies may be used in methods known within the art relating to the localization and/or quantitation of a SECX protein (*e.g.*, for use in measuring levels of the SECX protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies for SECX proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds [hereinafter "Therapeutics"].

An anti-SECX antibody (*e.g.*, monoclonal antibody) can be used to isolate SECX by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-SECX antibody can

facilitate the purification of natural SECX from cells and of recombinantly produced SECX expressed in host cells. Moreover, an anti-SECX antibody can be used to detect SECX protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the SECX protein. Anti-SECX antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

SECX Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding SECX protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively linked to the nucleic acid sequence to be expressed. Within a